

**AMENDMENT**

It is respectfully requested that the claims be amended without prejudice, without admission, without surrender of subject matter, and without any intention of creating any estoppel as to equivalents, as follows.

**In the Claims:**

1. (Currently Amended) A multilayer device for use in tissue engineering, comprising:
  - (a) at least a first layer comprised of a micromachined polymer scaffold suitable for attachment and culturing of animal cells and having a pattern of microchannels therein and
    - (i) wherein the microchannels are suitable for the attachment and culturing of animal cells within the microchannels, and
    - (ii) wherein the microchannels are connected for the circulation of fluid through the first layer, and
  - (b) at least a second layer comprised of a micromachined polymer scaffold suitable for attachment and culturing of animal cells,  
wherein the first and second layers are joined or fastened together and the first layer is formed by forming a mold from a substrate material using a photoresist processing technique, and casting the first layer on the respective mold.
2. (Cancelled)
3. (Cancelled)
4. (Cancelled)
5. (Previously presented) The multilayer device of claim 1, wherein the polymer of the first layer is biodegradeable.
6. (Previously presented) The multilayer device of claim 1, wherein the polymers of all the layers of the multilayer device are biodegradable.
7. (Previously presented) The multilayer device of claim 1, wherein the second layer has a pattern of microchannels therein.

8. (Previously presented) The multilayer device of claim 7, wherein the pattern in the first and second layers are similar.

9. (Previously presented) The multilayer device of claim 7, wherein the pattern in the first and second layers are different.

10. (Previously presented) The multilayer device of claim 7, wherein the pattern in the first layer is suitable for the culturing of endothelial cells and the layer in the second layer is suitable for the culturing of parenchymal cells.

11. (Previously presented) The multilayer device of claim 7, wherein the patterns of the first and second layers are aligned to form a vasculature.

12. (Previously presented) The multilayer device of claim 1, wherein the channels of the first layer are connected beginning from one or more inlets, expanding into more channels, and then converging back into one or more outlets.

13. (Previously presented) The multilayer device of claim 1, wherein the channels of all the layers of the multilayer device are connected beginning from one or more inlets, expanding into more channels, and then converging back into one or more outlets.

14. (Previously presented) The multilayer device of claim 1, further comprising a third layer comprised of a micromachined polymer scaffold suitable for attachment and culturing of animal cells, wherein the first, second and third layers are joined or fastened together.

15. (Previously presented) The multilayer device of claim 14,

(a) wherein the second layer is unpatterned; and

(b) wherein the third layer has a pattern of channels therein,

(i) wherein the channels are suitable for the attachment and culturing of animal cells within the channels, and

(ii) wherein the channels are connected for the circulation of fluid through the layer.

16. (Previously presented) The multilayer device of claim 15, wherein the pattern in the first and third layers are similar.

17. (Previously presented) The multilayer device of claim 15, wherein the pattern in the first and third layers are different.

18. (Previously presented) The multilayer device of claim 17, wherein the pattern in the first layer is suitable for the culturing of endothelial cells and the layer in the third layer is suitable for the culturing of parenchymal cells.

19. (Previously presented) The multilayer device of claim 1, wherein the animal cells are selected from the group consisting of endothelial cells, parenchymal cells, bone marrow cells, osteoblasts, mesenchymal stem cells, satellite cells, and fibroblasts.

20. (Previously presented) The multilayer device of claim 1, wherein the cells cultured in the channels of the first layer are endothelial cells.

21. (Previously presented) The multilayer device of claim 1, wherein one or more of the layers comprise through-holes.

22. (Previously presented) The multilayer device of claim 1, wherein one or more of the layers comprise an alignment indentation on the surface of a layer and an alignment protrusion on an opposing surface of a layer, the alignment indentations shaped to mate with the alignment protrusion.

23. (Previously presented) The multilayer device of claim 1, wherein the first layer is subdivided into zones of animal cell support.

24. (Previously presented) The multilayer device of claim 23, wherein the zones of animal cell support comprise cell adhesion molecules.

25. (Currently amended) A method of making a multilayer device, comprising the steps of:

- (a) obtaining at least a first layer comprised of a ~~micromachined~~ polymer scaffold suitable for attachment and culturing of animal cells and having a pattern of channels therein, wherein the polymer scaffold is approximately 10 to 500 microns thick, and
  - (i) wherein the channels are suitable for the attachment and culturing of animal cells within the channels, and
  - (ii) wherein the channels are connected for the circulation of fluid through the layer, [[:]] and
  - (iii) wherein the channels are about 2 to 50 microns in height and width;
- (b) obtaining at least a second layer for supporting animal cell growth wherein the second layer is comprised of a ~~micromachined~~ polymer scaffold suitable for attachment and culturing of animal cells; and
- (c) joining or fastening together the first and second layers.

26. (Previously presented) The method of claim 25, wherein the joining or fastening is by a method selected from the group consisting of the methods of solvent bonding; reflow by heating; treating the surface of the layer with oxygen plasma; polymer flow at the surface of the layer, mechanically fastening the layers with fasteners selected from the group comprising barbs, pins, screws, clamps, staples, wires, string, and sutures; and adhering the layers by the use of adhesives, adhesive films or adhesive layers.

27. (Withdrawn) A method of making a multilayer device, comprising the steps of:

- (a) obtaining a layer comprised of a material suitable for attachment and culturing of animal cells and having a pattern of channels therein,
  - (i) wherein the channels are suitable for the attachment and culturing of animal cells within the channels, and
  - (ii) wherein the channels are connected for the circulation of fluid through the layer; and
- (b) folding or rolling the layer to form a multilayer device having channels.

28. (Currently Amended) A method of making a multilayer device ~~containing animal cells~~, comprising the steps of:

(a) obtaining a multilayer device, comprising:

(i) at least a first layer comprised of a ~~micromachined~~ polymer scaffold suitable for attachment and culturing of animal cells and having a pattern of microchannels therein, and

(A) wherein the pattern of microchannels are suitable for the attachment and culturing of animal cells within the channels, ~~and~~

(B) wherein the pattern of microchannels are connected for the circulation of fluid through the layer; and

(C) wherein the first layer is formed by creating a microfluidic circuit pattern on a substrate by using a semiconductor manufacturing process, transferring the microfluidic circuit to an elastomer which acts as a mold for the polymer scaffold, and

(ii) at least a second layer, wherein the second layer is comprised of a ~~micromachined~~ polymer scaffold suitable for attachment and culturing of animal cells, wherein the first and second layers are fastened together, ~~and~~

(b) ~~adding, loading or seeding animal cells onto at least the first layer.~~

29. (Previously presented) The method of claim 28, wherein the animal cells are selected from the group consisting of endothelial cells, parenchymal cells, bone marrow cells, osteoblasts, mesenchymal stem cells, satellite cells, and fibroblasts.

30. (Previously presented) The method of claim 28, further comprising the step of (c) seeding animal cells into the channels.

31. (Previously presented) The method of claim 28, wherein the animal cells are endothelial cells.

32. (Currently Amended) A method of implanting a bioartificial organ into a recipient, comprising:

- (a) obtaining a multilayer device, comprising:
  - (i) at least a first layer comprised of a ~~micromachined~~ polymer scaffold suitable for attachment and culturing of animal cells and having a pattern of microchannels therein, and
    - (A) wherein the microchannels are suitable for the attachment and culturing of animal cells within the channels, and
    - (B) wherein the microchannels are connected for the circulation of fluid through the layer, and
    - (C) wherein the polymer scaffold is molded by optically creating a microfluidic pattern on a substrate which acts as a mold, casting the polymer scaffold on the mold, and removing the polymer scaffold from the mold; and
  - (ii) at least a second layer wherein the second layer is comprised of a ~~micromachined~~ polymer scaffold suitable for attachment and culturing of animal cells, the layers of the multilayer device being fastened together; ~~the multilayer device comprising animal cells;~~ and
- (b) implanting the multilayer device ~~comprising animal cells~~ into the recipient, wherein the implanted multilayer device ~~comprising animal cells~~ is a bioartificial organ.

33. (Withdrawn) An image reversal method for forming a scaffold having a material having a pattern of microchannels therein, comprising

- (a) selecting a mold having a complex pattern of microchannels on the mold in a reverse image of the channels; and
- (b) replica molding the pattern from the mold to a material suitable for attachment and culturing of animal cells;

wherein the replica molding forms a scaffold having a complex pattern of microchannels therein.

34. (Withdrawn) A multilayer device, comprising:
  - (a) multiple layers of tissue;
  - (b) multiple layers of material suitable for attachment and growth of tissue and having a pattern of microchannels in the material;
  - (b) vasculature within the tissue or on the material; and
  - (c) connections for flow into and out of the vasculature.
35. (Withdrawn) The multilayer device of claim 34, wherein the vasculature comprises endothelial cells.
36. (Withdrawn) The multilayer device of claim 35, further comprising cells selected from the group consisting of parenchymal cells, cells forming cartilage or bone, muscle cells, and nerve cells.
37. (Withdrawn) The multilayer device of claim 36, wherein the parenchymal cells are derived from organs selected from the group consisting of heart, liver, pancreas, intestine, kidney, reproductive tissues and lung.
38. (Previously presented) The multilayer device of claim 10 or 18, wherein the pattern in the first layer comprises microchannels that are about 10 to 50 microns in diameter.

Please add the following new Claims:

39. (New) The multilayer device of claim 1, wherein the substrate is selected from the group consisting of silicon, ceramic, and glass.
40. (New) The multilayer device of claim 1, wherein the second layer is formed by forming a mold from a substrate selected from the group consisting of silicon, ceramic, and glass using a photoresist processing technique, and casting the second layer on the respective mold.
41. (New) The multilayer device of claim 40, wherein the molds for the first and second layers are the same.
42. (New) The multilayer device of claim 40, wherein the molds for the first and second layers are different.
43. (New) The multilayer device of claim 1, wherein the polymer scaffold is selected from a material consisting of a biocompatible material, a biodegradable material, a porous material, a non-porous material and combinations thereof.

44. (New) The multilayer device of claim 1, wherein the microchannels are a branched pattern.
45. (New) The multilayer device of claim 1, wherein the microchannels are about 10 to 50 microns in height and width.
46. (New) The multilayer device of claim 1, wherein a resist coating in combination with the substrate material form the mold.
47. (New) The multilayer device of claim 1, wherein the mold is formed by etching the substrate.
48. (New) The multilayer device of claim 1, wherein the mold is formed by etching the substrate in a pattern exposed through resist and stripping the resist therefrom.
49. (New) The method of claim 25, wherein the lumens are substantially rectangular.
50. (New) The method of claim 25, wherein the first layer is formed by forming a mold from a substrate material selected from the group consisting of silicon, ceramic, and glass, using a thick resist processing technique, and casting the first layer on the respective mold.
51. (New) The method of claim 28, wherein the polymer scaffold is compression molded on the mold.
52. (New) The method of claim 28, wherein the polymer scaffold is about 200 microns thick.
53. (New) The method of claim 28, wherein the microchannels are about 2 microns in width.
54. (New) The method of claim 28, further comprising the steps of implanting the multilayer device and adding, loading or seeding animal cells onto at least the first layer
55. (New) The method of claim 32, wherein the elastomer is a PDMS elastomer.
56. (New) The method of claim 32, wherein the multilayer device is seeded with animal cells.
57. (New) The method of claim 56, wherein the multilayer device is seeded with animal cells after implanting.

58. (New) A multilayer device for use in tissue engineering, comprising:

- (a) at least a first layer comprised of a polymer scaffold suitable for attachment and culturing of animal cells and having a pattern of microchannels therein and
  - (ii) wherein the microchannels are suitable for the attachment and culturing of animal cells within the microchannels, and
  - (ii) wherein the microchannels are connected for the circulation of fluid through the first layer, and
- (b) at least a second layer comprised of a polymer scaffold suitable for attachment and culturing of animal cells,

wherein the first and second layers are joined or fastened together, about 10 to 500 microns thick, and the microchannels are about 2 to 50 microns in width.